

# Molecular Basis of Human Piebaldism

Richard A. Spritz

Departments of Medical Genetics and Pediatrics, University of Wisconsin, Madison, Wisconsin, U.S.A.

**Piebaldism is an autosomal dominant genetic disorder of pigmentation characterized by congenital patches of white skin and hair that lack melanocytes. Piebaldism results from mutations of the *KIT* proto-oncogene, which encodes the cell-surface receptor transmembrane tyrosine kinase for an embryonic growth factor, Steel factor. Several pathologic mutations of the *KIT* gene have now been identified in different**

**patients with piebaldism. Correlation of these mutations with the associated piebald phenotypes has led to the recognition of a hierarchy of three classes of mutations that result in a graded series of piebald phenotypes, and to improved understanding of the mechanisms that underlie dominant genetic disorders. Key words: *KIT*/receptor/tyrosine kinase/growth factor. *J Invest Dermatol* 103:137S-140S, 1994**

**P**iebaldism is an autosomal dominant genetic disorder of melanocyte development characterized clinically by congenital patches of white skin (leukoderma) and white hair (poliosis), principally located on the scalp, forehead, ventral chest and abdomen, and extremities [1-3]. Unlike vitiligo, with which it is frequently confused, in piebaldism the depigmented patches are present from birth and are generally static in shape and distribution, although limited filling in sometimes occurs, especially in milder cases. Hirschsprung disease (aganglionic megacolon) is occasionally also present, but most affected individuals display only the pigmentary anomaly. Piebaldism is relatively rare, although its actual frequency is not known. Because of its distinctive phenotype, piebaldism, sometimes incorrectly called partial albinism, has been known since at least ancient Greek times [4], and was one of the first autosomal dominant genetic disorders recognized [5]. Piebaldism was also one of the first genetic disorders for which a pedigree was presented, and several families have been reported that trace inheritance of the disorder over hundreds of years (reviewed in [2]). Etymologically, pie apparently refers to the variegated black and white plumage pattern characteristic of the magpie, and bald derives from the Greek *phalios*, having a white spot. Histologically, the depigmented patches lack most or all melanocytes [6,7], although areas of increased pigmentation may occur at the boundaries of or even within the regions of hypopigmentation. Melanocytes derive embryologically from the neural crest, and piebaldism has thus been considered to be a lineage-specific disorder of neural crest development, most likely involving defective melanoblast proliferation, migration, or survival.

The critical clues to elucidation of the molecular basis of human piebaldism came from studies of a similar disorder of mice, dominant white spotting (*W*). Mouse dominant white spotting, which is associated with defects of pigmentation, hematopoiesis, and germ-cell development (reviewed in [8,9]), was found to result from deletions or point mutations of the *c-kit* protooncogene (reviewed in [10]). The *c-kit* gene, originally identified in the genome of the HZ4-feline sarcoma virus [11], encodes the cell-surface receptor for an embryonic growth factor. This growth factor, which has been

variously called mast cell growth factor, stem cell factor, KL (kit ligand), or Steel factor (SLF) (reviewed in [10]), is the product of the steel (*Sl*) locus of mice, and both *Sl* and *W* mutant mice display similar piebald-like phenotypes.

The human piebaldism locus was tentatively mapped to chromosome segment 4q12 on the basis of several patients with piebaldism associated with *de novo* chromosomal translocations or deletions involving this region [12-15]. Subsequently, the human *KIT* gene was mapped to approximately the same chromosomal location [16,17], suggesting that human piebaldism might, like mouse dominant white spotting, also result from abnormalities of the *KIT* gene. Our confirmation of this hypothesis, the subsequent identification of a variety of pathologic mutations of the *KIT* gene in different families with piebaldism, and the correlation of these specific mutations with the resultant clinical phenotypes has proved highly instructive of the biology of the Steel-*KIT* system of signal transduction.

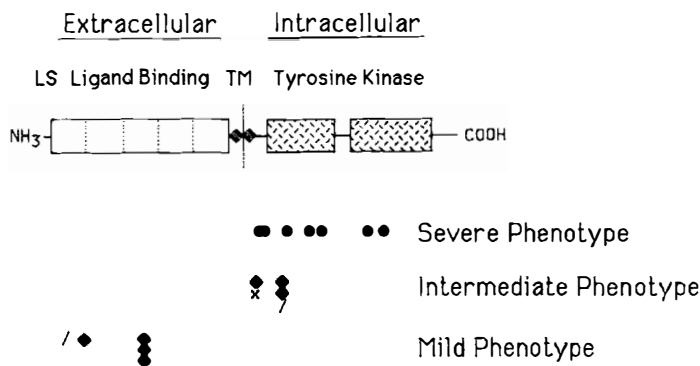
## BIOLOGY OF THE KIT RECEPTOR

The *KIT* protein is a member of the type III group of transmembrane receptor tyrosine kinases. As illustrated in **Fig 1**, the *KIT* polypeptide consists of an amino-terminal extracellular ligand-binding receptor domain composed of five immunoglobulin-type repeats, a short transmembrane domain, and an intracellular domain consisting of a bipartite tyrosine kinase domain followed by a carboxy-terminal tail. The stoichiometry of interaction between Steel factor and the extracellular ligand-binding domain of the *KIT* receptor is not certain, but is thought to be monovalent [18]. However, on binding the Steel factor ligand, the *KIT* receptor undergoes dimerization within the cell membrane [18] (reviewed in [10]), activating its intracellular tyrosine kinase. This, in turn, results in autophosphorylation of specific tyrosine residues within the *KIT* kinase domain, enhancing the binding of various proteins, including phosphatidylinositol 3' kinase and phospholipase C $\gamma$ 1, which act as downstream mediators of the mitogenic signal in the *KIT*-dependent pathway of signal transduction (reviewed in [10]).

The human *KIT* structural gene consists of 21 exons distributed over more than 70 kb (kilobases) at chromosome segment 4q12 [19,20], adjacent to the closely related *PDGFRA* and *KDR* genes, which encode similar type III receptor tyrosine kinases for different polypeptide growth factors. The first exon of the *KIT* gene encodes the translational initiation codon and an apparent signal peptide [19], and the last exon principally encodes the very long 3'-untrans-

Reprint requests to: Dr. Richard A. Spritz, Departments of Medical Genetics and Pediatrics, 317 Laboratory of Genetics, 445 Henry Mall, University of Wisconsin, Madison, WI 53706.

Abbreviations: SSCP, single-stranded conformation polymorphism; YAC, yeast artificial chromosome.



**Figure 1. Schematic diagram of the human KIT polypeptide and both published and unpublished mutations that are associated with human piebaldism.** The amino-terminal extracellular leader sequence (LS) and penta-repetitive ligand binding domain, the transmembrane domain (TM), and the intracellular bi-partite tyrosine kinase domain are indicated. Missense amino acid substitutions are indicated by solid circles, frame-shifts by solid diamonds, splice junction mutations by diagonal slashes, and a nonsense mutation by X.

lated region of *KIT* mRNA. The *KIT* mRNA precursor is alternatively spliced at exon 9, resulting in the production of a minor 976-amino acid *KIT* polypeptide and a predominant 972-amino acid polypeptide lacking residues 510 to 513 [19]. The same general gene structure [21] and alternative splicing of *KIT* mRNA also occur in the mouse, and the two alternative isoforms of the mouse *KIT* receptor exhibit distinctly different properties in the absence of exogenous Steel factor [22]. The intracellular tyrosine kinase domain is the most highly evolutionarily conserved segment, both within the *KIT* polypeptide itself and among a wide variety of different tyrosine kinases from a diverse array of species. As expected, *KIT* mRNA is expressed by human melanocytes, although expression of *KIT* appears to frequently be down-regulated in melanomas [23]. The *KIT* receptor is also expressed in brain, placenta, gut, erythroid stem cells of the bone marrow, mast cells, breast, and in the gonads.

#### MUTATION OF THE *KIT* GENE IN HUMAN PIEBALDISM

To test the hypothesis that human piebaldism might result from abnormalities of the *KIT* gene, we first studied a patient with piebaldism, mental retardation, and multiple congenital anomalies associated with a *de novo* interstitial deletion of segment q12-q21.1 of chromosome 4 [15]. By means of quantitative Southern blot hybridization analyses we showed that this deletion included both the *KIT* and adjacent *PDGFRA* genes [24]. Thus, this patient was hemizygous for a chromosomal segment that spans both of these loci. Fleischman and coworkers [25] likewise described a similar patient with deletion of both the *KIT* and *PDGFRA* genes, although this deletion was not detectable cytogenetically. The deletions in these two patients thus appeared homologous to the *W<sup>19H</sup>* deletion of mouse, which also includes both the *KIT* and *PDGFRA* genes, and strongly supported the hypothesis that human piebaldism and mouse *W* are homologous.

To determine whether human piebaldism can also result from point mutations of the *KIT* gene, we defined the organization and nucleotide sequence of the human *KIT* gene and characterized its DNA sequence in a series of patients with typical autosomal dominant piebaldism. To do this, we amplified all 21 exons of the *KIT* gene from genomic DNA by use of the polymerase chain reaction (PCR) and initially subjected the amplified products to complete DNA sequence analysis. More recently, we have utilized PCR-based single-stranded conformation polymorphism (SSCP)/heteroduplex analysis to localize mutations to specific *KIT* exons prior to DNA sequencing. As shown in Fig 1, 10 different point mutations of the *KIT* gene have now been reported in different families with piebaldism, as well as several additional mutations not yet pub-

lished. These mutations can be classified into three general groups, each group tending to be associated with piebald phenotypes of differing severity. Thus, a hierarchical paradigm of pathologic human *KIT* mutations appears to account for a graded series of dominant phenotypes in human piebaldism.

The first group of *KIT* gene mutations consists of missense substitutions that result in amino acid substitutions, all of which are located within the intracellular tyrosine kinase domain. Five such mutations have been reported, Glu583Lys [26], Phe584Leu [27], Gly664Arg [28], Arg791Gly [29], and Gly812Val [29], almost all involving amino acid residues that have been especially highly conserved during mammalian evolution. Moreover, the locations of several of these human *KIT* missense substitutions correspond very closely to the positions of similar mutations that have been identified in various strains of *W* mutant mice (reviewed in [10]) (the human Glu583Lys mutation corresponds precisely to the mouse *W<sup>37</sup>* mutation), providing further evidence of the importance of these spots to function of the *KIT* receptor. These five human *KIT* missense substitutions are all associated with relatively severe piebald phenotypes. This is a consequence of the fact that the *KIT* kinase is only activated on dimerization of the receptor. In patients heterozygous for *KIT* missense mutations one-fourth of the *KIT* receptor dimers contain only the abnormal *KIT* polypeptide, which are inactive. However, half of the *KIT* receptor dimers consist of one normal *KIT* polypeptide and one abnormal *KIT* polypeptide; these heterodimers are also inactive. Thus, patients heterozygous for these so-called dominant-negative *KIT* missense mutations have only one-fourth of the normal amount of *KIT* receptor, and accordingly they exhibit relatively severe piebald phenotypes.

In contrast, the second group of *KIT* mutations completely eliminate the production of *KIT* protein by the mutant gene. Patients heterozygous for these so-called loss of function mutant alleles thus express half of the normal amount of *KIT* receptor. Apparently, however, half of the normal amount of receptor is not adequate, resulting in haploinsufficiency for *KIT*-dependent signal transduction. These *KIT* mutations are thus associated with relatively mild piebald phenotypes, in which the depigmented patches are usually small and poliosis is often absent, although affected individuals frequently experience early graying of the hair. In fact, some members of families with *KIT* mutations of this type have been so mildly affected that the clinical diagnosis was only made subsequent to DNA diagnosis. We have described two such different mutations, both frameshifts located very proximal in the *KIT* gene, at codon 85 [30] and at codons 250–251 [31]. Interestingly, we identified the same codon 250–251 frameshift in three different, unrelated families with mild piebaldism [31]. This apparently recurrent *KIT* gene mutation may account for a significant fraction of human piebaldism, especially among clinically milder cases.

The third group of patients with piebaldism exhibit a very variable phenotype, ranging from extremely mild to quite severe, even among affected members of an individual family. We have described three *KIT* mutations of this type, two frameshifts [27] and a splice junction mutation [30], which would all result in premature termination of translation, truncating the nascent *KIT* polypeptide distally, within the intracellular tyrosine kinase domain. Clearly, these mutations abolish expression of normal *KIT* polypeptide from this allele. However, the truncated *KIT* receptor apparently can still bind Steel factor and even form dimers, dominant negatively inhibiting function of the normal *KIT* polypeptide [18]. It is likely, however, that both the truncated *KIT* polypeptides and the incompletely translated *KIT* mRNA are relatively unstable. Therefore, these mutations probably reduce *KIT* receptor function to an amount between one-fourth and half of normal, accounting for the intermediate and highly variable piebald phenotype.

Interestingly, the only amino acid substitution we have observed within the extracellular ligand-binding domain of the *KIT* receptor appears to be a rare non-pathologic variant (unpublished data). As noted above, this region consists principally of five immunoglobulin-like domains, and it is possible that this segment may be at least in part functionally redundant. Thus, many amino acid substitu-

tions in this portion of the KIT polypeptide may have little or no effect on ligand binding, and thus may have little or no phenotypic effect. However, the extracellular portion of the KIT polypeptide is also thought to contain the segments that mediate receptor dimerization, and it seems likely that pathologic amino acid substitutions of residues involved in this process may be encountered in the future.

Thus, piebaldism is associated with reduced expression of the KIT receptor, resulting in decreased KIT-dependent signal transduction and abnormal distribution of melanoblasts during embryologic development. Recent evidence indicates that reduced expression of KIT likely results in decreased proliferation of melanoblasts during embryologic development [32].

#### ARE THERE OTHER HUMAN PIEBALDISM GENES?

Overall, we have been able to identify pathologic point mutations of the KIT gene in approximately two-thirds of the more than thirty unrelated patients with typical piebaldism we have studied to date, and several more appear to have deletions that include part of all of KIT (unpublished data). However, in about one-fourth of patients we can find no apparent abnormalities of the KIT gene. Some of these patients may have occult KIT gene mutations not detected by SSCP/heteroduplex screening; however, in at least several of these patients we have also not found any abnormalities by complete DNA sequence analysis. Interestingly, none of these patients have abnormalities of the MGF gene (the formal name for the human Steel factor gene), suggesting that, in contrast with mice, mutations of this gene may not result in the piebald phenotype in humans. Furthermore, we have conducted genetic linkage analyses in several of these families, and in all of these the piebald trait was completely linked to the KIT gene (unpublished data). Together, these data suggest that most or all of these patients either have occult mutations (or deletions) in the KIT gene not detected by our analyses or that they have mutations in a nearby gene also required for normal developmental patterning of melanocytes. What might constitute a second human piebaldism gene in this region? We have recently isolated and mapped a contig of human recombinant yeast artificial chromosomes (YACs) spanning the entire human PDGFRA-KIT-KDR type III receptor tyrosine kinase gene cluster, covering more than 2 megabases of DNA in chromosome segment 4q12 [33]. In mouse, the patch (Ph) mutation, which results in a dominant white spotting phenotype similar to that of W, comprises a deletion that includes the pdgfra gene but not c-kit [24,35]. It is not yet clear whether white spotting in patch mice results from deletion of the pdgfra gene itself or from inhibitory effects of the large chromosomal deletion on expression of the nearby c-kit gene. Thus, it remains possible that some cases of human piebaldism may result from mutations in the PDGFRA gene or other genes in this region that have yet to be identified.

#### RELATED PROBLEMS

In addition to piebaldism, abnormalities of the Steel factor-KIT pathway of signal transduction have been implicated in two other disorders. Longley and coworkers [36] recently reported aberrant expression of the Steel factor ligand in patients with cutaneous mastocytosis. This disorder is associated with both mast cell proliferation and increased skin pigmentation, and it is likely that both of these symptoms result from increased or inappropriate stimulation of the Steel-KIT-dependent pathway of signal transduction. Abnormalities of the Steel-KIT system have also been sought in patients with Diamond-Blackfan anemia, a heterogeneous group of clinically severe congenital hypoplastic anemias. In addition to characteristic white patches, W mutant mice with c-kit mutations frequently exhibit a hypoplastic anemia similar to that of humans with Diamond-Blackfan anemia. However, humans with piebaldism do not have anemia [37], and several investigators have failed to find abnormalities of either the KIT or MGF genes in patients with Diamond-Blackfan anemia [38–40]. Thus, these genes no longer are viable candidate genes for this disorder.

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#### REFERENCES

- Keeler CE: The heredity of a congenital white spotting in Negroes. *J Am Med Assoc* 103:179–180, 1934
- Froggatt P: An outline with bibliography of human piebaldism and white forelock. *Ir J Med Sci* 398:86–94, 1951
- Cooke JV: Familial white skin spotting (piebaldness) ('partial albinism') with white forelock. *J Pediatr* 41:1–12, 1952
- Lucian: *The works of Lucian of Samosata*, Vol 1. Clarendon, Oxford, 1905
- Morgan J: Some accounts of motley colored or pye Negro girl and mulatto boy. *Trans Am Philos Soc* 2:392–395, 1786
- Breathnach AS, Fitzpatrick TB, Wyllie LMA: Electron microscopy of melanocytes in a case of human piebaldism. *J Invest Dermatol* 45:2–37, 1965
- Jimbow K, Fitzpatrick TB, Szabo G, Hori Y: Congenital circumscribed hypomelanosis: a characterization based on electron microscopic study of tuberous sclerosis, nevus depigmentosus, and piebaldism. *J Invest Dermatol* 64:50–62, 1975
- Silvers W: *The Coat Colors of Mice*. Springer Verlag, New York, 1979
- Lyon MF, Searle AG: *Genetic Variants and Strains of the Laboratory Mouse*. Oxford University Press, New York, 1989
- Morrison-Graham K, Takahashi Y: Steel factor and c-Kit receptor: from mutants to a growth factor system. *BioEssays* 15:77–83, 1993
- Besmer P, Murphy JE, George PC, Qiu F, Bergold PJ, Lederman L, Snyder HW Jr, Brodeur D, Zuckerman EE, Hardy WD: A new acute transforming feline retrovirus and the relationship of its oncogene v-kit with the protein kinase gene family. *Nature* 320:415–421, 1986
- Funderburk SJ, Crandall BF: Dominant piebald trait in a retarded child with a reciprocal translocation and small intercalary deletion. *Am J Hum Genet* 26:715–722, 1974
- Lacassie Y, Thurmon TF, Tracy MC, Pelias MZ: Piebald trait in a retarded child with interstitial deletion of chromosome 4. *Am J Hum Genet* 29:641–642, 1977
- Hoo JJ, Haslam RHA, van Orman C: Tentative assignment of piebald trait to chromosome band 4q12. *Hum Genet* 73:230–231, 1986
- Yamamoto Y, Nishimoto H, Ikemoto S: Interstitial deletion of the proximal long arm of chromosome 4 associated with father-child incompatibility within the Gc-system: probable reduced gene dosage effect and partial piebald trait. *Am J Med Genet* 32:520–523, 1989
- Yarden Y, Kuang W-J, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, Chen E, Schlessinger J, Francke U, Ullrich A: Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. *EMBO J* 6:3341–3351, 1987
- d'Auriol L, Mattei MG, Andre C, Galibert F: Localization of the human c-kit protooncogene on the q11-q12 region of chromosome 4. *Hum Genet* 78:374–376, 1988
- Lev S, Yarden Y, Givol D: Dimerization and activation of the kit receptor by monovalent and bivalent binding of the stem cell factor. *J Biol Chem* 267:15970–15977, 1992
- Giebel LB, Strunk KM, Holmes SA, Spritz RA: Organization and nucleotide sequence of the human KIT (mast/stem cell growth factor receptor) proto-oncogene. *Oncogene* 7:2207–2217, 1992
- Vandenbark GR, DeCastro CM, Taylor H, Dew-Knight S, Kaufman RE: Cloning and structural analysis of the human c-kit gene. *Oncogene* 7:1259–1266, 1992
- Gokkel E, Grossman Z, Ramot B, Yarden Y, Rechavi G, Givol D: Structural organization of the murine c-kit proto-oncogene. *Oncogene* 7:1423–1429, 1992
- Reith AD, Ellis C, Lyman SD, Anderson DM, Williams DE, Bernstein A, Pawson T: Signal transduction by normal isoforms and W mutant variants of the Kit receptor tyrosine kinase. *EMBO J* 10:2451–2459, 1991
- Natali PG, Nicotra MR, Winkler AB, Cavaliere R, Bigotti A, Ullrich A: Progression of human cutaneous melanoma is associated with loss of expression of c-kit proto-oncogene receptor. *Int J Cancer* 52:197–201, 1992
- Spritz RA, Doroetto S, Fukushima Y: Deletion of the KIT and PDGFRA genes in a patient with piebaldism. *Am J Med Genet* 44:492–495, 1992
- Fleischman RA, Saltman DL, Stastny V, Zneimer S: Deletion of the c-kit protooncogene in the human developmental defect piebald trait. *Proc Natl Acad Sci USA* 88:10885–10889, 1991
- Fleischman RA: Human piebald trait resulting from a dominant negative mutant allele of the c-kit membrane receptor gene. *J Clin Invest* 89:1713–1717, 1992
- Spritz RA, Giebel LB, Holmes SA: Dominant negative and loss of function mutations of the c-kit (mast/stem cell growth factor receptor) proto-oncogene in human piebaldism. *Am J Hum Genet* 50:261–269, 1992
- Giebel LB, Spritz RA: Mutation of the KIT (mast/stem cell growth factor receptor) protooncogene in human piebaldism. *Proc Natl Acad Sci USA* 88:8696–8699, 1991
- Spritz RA, Holmes SA, Itin P, Küster W: Novel mutations of the KIT (mast/stem cell growth factor receptor) proto-oncogene in human piebaldism. *J Invest Dermatol* 101:22–25, 1993
- Spritz RA, Holmes SA, Ramesar R, Greenberg J, Curtis D, Beighton P: Mutations

- of the *KIT* (mast/stem cell growth factor receptor) proto-oncogene account for a continuous range of phenotypes in human piebaldism. *Am J Hum Genet* 51:1058-1065, 1992
31. Spritz RA, Holmes SA, Berg SZ, Nordlund JJ, Fukui K: A recurrent deletion in the *KIT* (mast/stem cell growth factor receptor) proto-oncogene is a frequent cause of human piebaldism. *Hum Molec Genet* 2:1499-1500, 1993
  32. Spritz RA, Ho L, Strunk KM: Inhibition of proliferation of human melanocytes by a *KIT* antisense oligodeoxynucleotide: implications for human piebaldism and mouse dominant white spotting (*W*). *J Invest Dermatol* (in press)
  33. Spritz RA, Lu-Kuo JM, Le Paslier DL, Althers MR, Dorman T, Moir DT, Strunk KM, Ward DC: A 2-Mb YAC contig spanning a cluster of genes encoding type III receptor tyrosine kinases (*PDGFRA-KIT-KDR*) is located at 4q12. *Genomics* 72:431-436, 1994
  34. Stephenson DA, Mercola M, Anderson E, Wang C, Stiles CD, Bowen-Pope DF, Chapman VM: Platelet-derived growth factor receptor  $\alpha$ -subunit gene (*Pdgfra*) is deleted in the mouse patch (*Ph*) mutation. *Proc Natl Acad Sci. USA* 88:6-10, 1991
  35. Smith EA, Seldin MF, Martinez L, Watson ML, Choudhury GG, Lalley PA, Pierce J, Aaronson S, Barker J, Naylor SL, Sakaguchi AY: Mouse platelet-derived growth factor receptor  $\alpha$  gene is deleted in *W<sup>1981</sup>* and patch mutations on chromosome 5. *Proc Natl Acad Sci USA* 88:4811-4815, 1991
  36. Longley J Jr, Morganroth GS, Tyrrell L, Ding TG, Anderson DM, Williams DE, Halaban R: Altered metabolism of mast-cell growth factor (*c-kit* ligand) in cutaneous mastocytosis. *N Engl J Med* 238:1302-1307, 1993
  37. Spritz RA: Lack of apparent hematologic abnormalities in human patients with *c-kit* (stem cell factor receptor) gene mutations. *Blood* 79:2497-2498, 1992
  38. Abkowitz JL, Broudy VC, Bennett LG, Zsebo KM, Martin FH: Absence of abnormalities of *c-kit* or its ligand in two patients with Diamond-Blackfan anemia. *Blood* 79:25-28, 1992
  39. Drachtman RA, Geissler EN, Alter BP: The SCF and *c-kit* genes in Diamond-Blackfan anemia. *Blood* 79:2177-2178, 1992
  40. Spritz RA: Lack of mutations of the *MGF* and *KIT* genes in Diamond-Blackfan anemia. *Blood* 81:3165, 1993